

- 44 -

Claims

1. An isolated polynucleotide molecule derivable from a polynucleotide encoding a polypeptide having L-sorbose dehydrogenase activity comprising a partial nucleotide sequence of at least 20 consecutive nucleotides of SEQ ID NO:1.
- 5 2. The isolated polynucleotide molecule according to claim 1, wherein the partial nucleotide sequence of SEQ ID NO:1 has at least 50 consecutive nucleotides.
3. The isolated polynucleotide molecule according to claim 1, wherein the partial nucleotide sequence of SEQ ID NO:1 has at least 100 consecutive nucleotides.
4. The isolated polynucleotide according to claim 3 wherein the partial nucleotide  
10 sequence is derivable from a polynucleotide sequence having a homology of at least 60% with SEQ ID NO:1 whereby at least 100 consecutive nucleotides are compared.
5. The isolated polynucleotide molecule according to any one of claims 1 to 4, whereby the partial nucleotide sequence is derivable from a polynucleotide sequence having a homology of at least 80% with SEQ ID NO:1.
- 15 6. The isolated polynucleotide molecule according to any one of claims 1 to 5, whereby the partial nucleotide sequence is derivable from a polynucleotide sequence having a homology of at least 90% with SEQ ID NO:1.
7. The isolated polynucleotide molecule according to any one of claims 1 to 6, which is selected from the group consisting of SEQ NOs:1, 11, 13, 15, 17, 19, 21 and 26.
- 20 8. The isolated polynucleotide molecule according to claim 1, wherein the partial nucleotide sequence is selected from the group consisting of SEQ ID NOs:5, 6, 7, 8, 9, 10, 23, and 24.
9. A polypeptide encoded by a polynucleotide according to any of the preceding claims.
- 25 10. The polypeptide according to claim 9, comprising a partial amino acid sequence of at least 25 consecutive amino acids selected from the group consisting of SEQ ID NO:2, 12, 14, 16, 18, 20, 22, and 27.
11. The polypeptide according to claim 9 or 10, wherein the partial amino acid sequence has at least 35 consecutive amino acids.

12. A recombinant DNA molecule for expression of a polypeptide having L-sorbose dehydrogenase activity, said recombinant DNA molecule comprising a polynucleotide according to any one of claims 1 to 7.
13. An expression vector comprising the recombinant DNA molecule according to  
5 claim 12.
14. A recombinant organism which has been transformed with the recombinant DNA according to claim 12 and/or the expression vector of claim 13.
15. The recombinant organism according to claim 14, wherein the recombinant DNA is at least partially integrated into the chromosome.
- 10 16. The recombinant organism according to claim 14 or 15, which is selected from the group consisting of fungal, plant, animal and bacterial cells.
17. The recombinant organism according to claim 16, wherein the organism is a bacterium of a genus selected from the group consisting of *Gluconobacter*, *Acetobacter*, *Pseudomonas* and *Escherichia*.
- 15 18. A process for the production of L-ascorbic acid from a substrate selected from D-sorbitol, L-sorbose and L-sorbose comprising:
- (a) propagating a recombinant organism of any one of claims 14 to 17 in an appropriate culture medium and
- (b) recovering and separating L-ascorbic acid from said culture medium.
- 20 19. A process for the production of L-ascorbic acid from a substrate selected from D-sorbitol, L-sorbose and L-sorbose comprising:
- (a) propagating a non-recombinant microorganism encoding a polypeptide according to claim 9 in an appropriate culture medium and
- (b) recovering and separating L-ascorbic acid from said culture medium.
- 25 20. A process for the production of L-ascorbic acid comprising contacting a substrate selected from D-sorbitol, L-sorbose and L-sorbose with the isolated polypeptide of claim 9.
21. A process for the production of L-ascorbic acid from a substrate selected from D-sorbitol, L-sorbose and L-sorbose comprising:

- 46 -

(a) propagating a recombinant of organism according to any one of claims 14 to 17 or a non-recombinant microorganism encoding the polypeptide according to claim 9 in an appropriate culture medium,

(b) isolating and purifying the L-sorbose dehydrogenase,

5 (c) incubating the substrate in the presence of the L-sorbose dehydrogenase of (b), and

(d) recovering and separating L-ascorbic acid from the reaction mixture.

22. A process for the production of L-sorbose dehydrogenase, wherein a recombinant organism comprising a polynucleotide according to any one of claims 1 to 7  
10 is propagated in an appropriate culture medium, the cells are disrupted and the L-sorbose dehydrogenase is isolated.

23. A process for the production of L-sorbose dehydrogenase, wherein a non-recombinant microorganism comprising a polynucleotide according to any one of claims 1 to 7 is propagated in an appropriate culture medium, the cells are disrupted and the L-  
15 sorbose dehydrogenase is isolated.

24. A process for the production of vitamin C comprising converting a substrate into vitamin C in a medium comprising resting cells of a microorganism.

25. The process according to claim 25 comprising the steps of:

(a) culturing the microorganism under conditions which enable growth,

20 (b) changing of the conditions such that the growth rate of the microorganism is reduced leading to resting cells; and

(c) production of vitamin C from the substrate using the resting cells of (b).

26. The process according to claim 25 wherein steps (a) and (c) are performed in 2 or more separate vessels.

25 27. The process according to claim 25 wherein step (a) and (c) are not separated by any washing and/or isolation step.

28. The process according to any one of claims 24 to 27 wherein the microorganism is grown in batch, fed-batch, continuous, or semi-continuous mode.

- 47 -

29. The process according to claim 25 wherein step (c) is performed in batch, fed-batch, continuous, or semi-continuous mode.
30. The process according any one of claims 24 to 29 wherein the density of the resting cells in the medium measured as OD at 600 nm is at least 10.
- 5 31. The process according to any one of claims 24 to 30 wherein the yield of produced vitamin C is at least 1.8 g/l.
32. The process according to any one of claims 24 to 31 wherein the microorganism is selected from the group consisting of yeast, algae, and bacteria.
33. The process according to any one of claims 24 to 32 wherein the microorganism is  
10 selected from the group consisting of *Candida*, *Saccharomyces*, *Zygosaccharomyces*, *Scyzosaccharomyces*, *Kluyveromyces*, *Chlorella*, *Gluconobacter*, *Acetobacter aceti*, *Pantoea*, *Cryptococcus*, *Pseudomonas* and *Escherichia*.
34. The process according to any one of claims 24 to 33 wherein the substrate is  
15 selected from the group consisting of D-glucose, D-sorbitol, L-sorbose, L-sorbose, 2-keto-L-gulonate, D-gluconate, 2-keto-D-gluconate and 2,5-diketo-gluconate.
35. The process according to any one of claims 24 to 34 using a microorganism capable of producing both vitamin C and 2-keto-L-gulonic acid from a substrate and wherein the ratio between the concentration of vitamin C and 2-KGA is more than 0.1.
36. The process according to any one of claims 18 to 21 or 24 to 35 further comprising  
20 isolation of vitamin C from the medium and optionally one or more purification steps.
37. The process according to claim 36 wherein all purification steps are performed in an aqueous environment.

\*\*\*